COMMENTARY

NEWS & VIEWS

Section through a human atherosclerotic carotid artery (page 996).

ARTICLES

Degeneration of cap:
around the retinal opti·
of rat pups expos
hyperoxia (page 1⌄

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The dark side of glucose

N n-enzymatic glycation, cellular receptors and oxidant stress together have implications for the path genesis f cellular dysfunction in diabetes and beyond (pages 1057–1061).

ANN MARIE SCHMIDT,
SHI DU YAN &
DAVID M. STERN

Proteins or lipids exposed to reducing sugars become non-enzymatically glycated and oxidized. Initially, reversible early glycation adducts form, the best known of which is haemoglobin A_{1c} (used for long-term monitoring of glucose control in patients with diabetes). Following complex molecular rearrangements, the irreversible advanced glycation end products (AGEs) develop. These constitute a heterogeneous class of structures of yellow-brown color, with a propensity to form crosslinks, that generate reactive oxygen intermediates and interact with particular cell surface structures. Although the yellow-brown glucose-modified structures were long recognized in food chemistry for their role in spoilage (the Maillard reaction), it was the insight of Anthony Cerami in 1977 that led to considering the pathobiological implications of non-enzymatic glycation. Work described in this issue of *Nature Medicine* by Li *et al.*[1] continues to build on Cerami's fundamental insight.

When does non-enzymatic glycation occur *in vivo*? Conditions favouring AGE formation include those in which protein and/or lipid turnover is prolonged or delayed on lysine-rich structures, especially in the setting of elevated levels of aldose and aldose phosphates. Such conditions occur during normal ageing, are accelerated in diabetes[2] and are also relevant to the pathogenesis of disorders characterized by amyloid formation and protein deposition, such as haemodialysis-associated amyloidosis and Alzheimer's disease[3,4]. For example, in Alzheimer's disease AGE-modification occurs on components of the intracellular neurofibrillary tangles[4,5], consistent with accelerated glycation under conditions of high concentrations of intracellular aldose phosphates[2], and in extracellular amyloid-β peptide accumulations[6,7]. Furthermore, AGE formation can occur even in the euglycemic state, as in hypercholesterolemic rabbits without diabetes, in which macromolecules are trapped in the expanded neointima of the atherosclerosis-prone animals[8].

AGE-modification of proteins and lipids has important biological implications. Long-lived proteins in the extracellular matrix are targets of non-enzymatic glycation, and the resulting crosslinked and protease-resistant structures promote trapping of macromolecules and accumulation of insoluble aggregates. The presence of AGEs in the matrix also modifies cellular interactions, as ᵥ mononuclear phagocytes. Whereas s ble AGEs promote monocyte migra down a concentration gradient, the p ence of immobilized AGEs arrests mc cytes, resulting in their subsequ sustained activation[9,10]. In the contex the vessel wall in diabetes, endotheli and smooth muscle cells are in intim contact with both basement membra and plasma components, constituting AGE-rich milieu (Fig. 1*a*, *b*). The sa considerations apply to mesangial ce embedded in a matrix abundant wi AGEs. It is evident that the impact AGEs on cellular properties is a major c terminant of their potential contributi to organ dysfunction.

How do AGEs perturb c llular fun tions? AGEs act on cells as a cons quence of their recognition of ce surface polypeptides on target cell Several binding sites for AGEs have bee identified[11-13]; the best characterized c these to date is a member in the im munoglobulin superfamily terme Receptor for AGE or RAGE[12]. This recep tor mediates monocyte migration and ac tivation in response to AGEs, althoug other binding sites may also con tribute[11,13]. Engagement by AGE of en dothelial RAGE induces cellular oxidan stress[10], thereby increasing vascular per meability and activating the transcription factor NF-κB; one consequence o the latter event is cell surface expression of vascular cell adhesion m lecule-1 (VCAM-1)[14]; it is noteworthy in this context that this adherence molecule is associated with the early phase of experimental atherosclerosis[15]. In the vasculature, RAGE is upregulated in a range of vasculopathies, in atherosclerotic vascular lesions (Fig. 1*c*, *d*), and in immune-inflammatory vasculitides. An intriguing aspect of the biology of RAGE is its high level of expression in developing brain, in which we have hypothesized that it interacts with ligand(s) distinct from AGEs. In fact, we have identified the neurite-promoting polypeptide amphoterin, which is co-expressed with the receptor in neonatal brain, as a ligand f RAGE[16]. It is known that a single receptor can recognize and bind to more than one biologi-

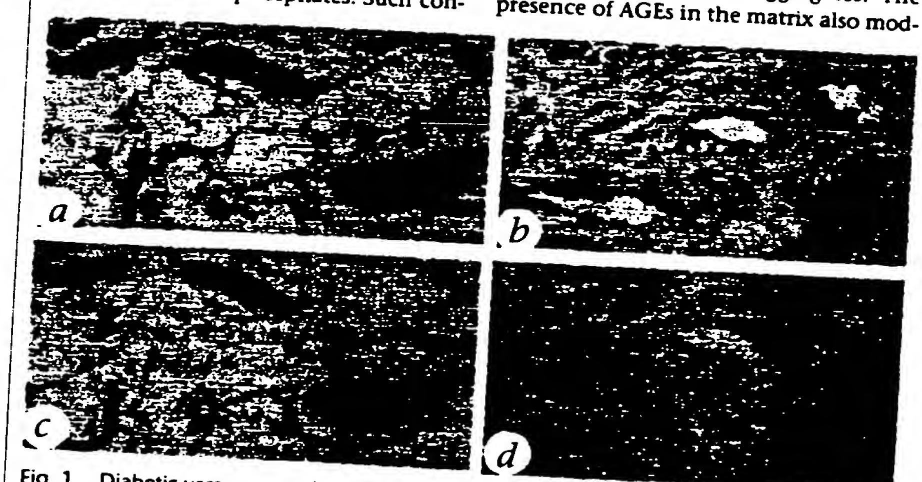

Fig. 1 Diabetic versus normal vasculature: AGE and RAGE. Co-localization of AGE antigen (*a*) and RAGE epitopes (*c*) in adjacent sections of diabetic vasculature (*a*, *c*) versus their absence in age-matched control vasculature (*b* and *d*, respectively, for AGE and RAGE). Affinity-purified anti-AGE IgG and anti-RAGE IgG were used to visualize their respective antigens[9]. Magnificati n, ×260.

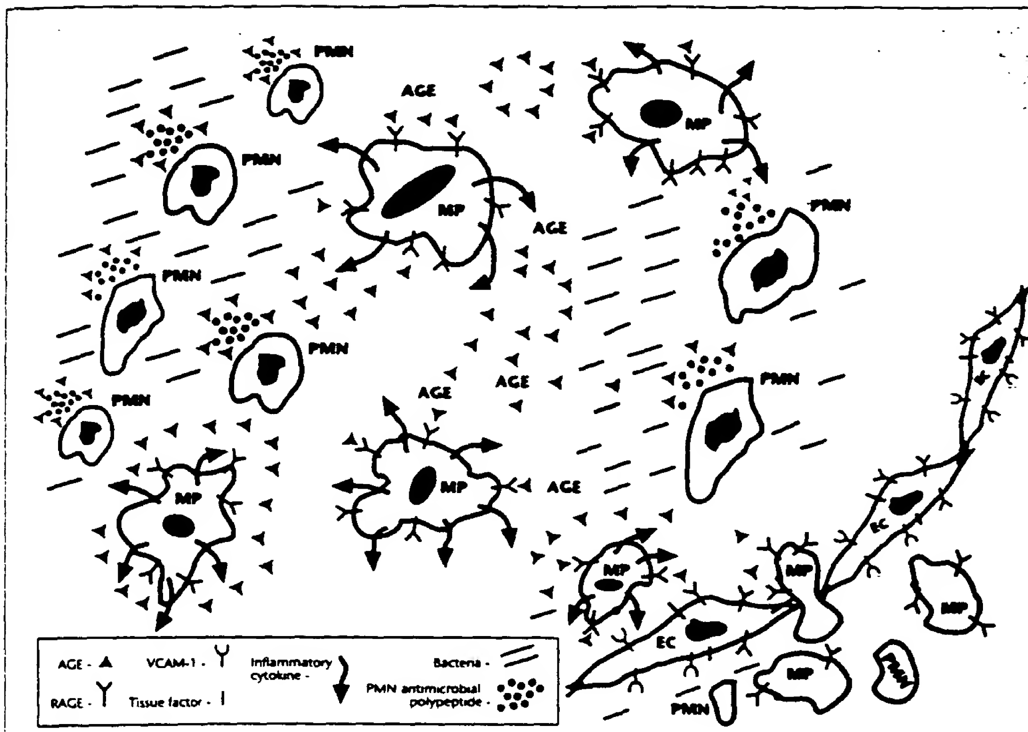

Fig. 2 Schematic depiction of the environmental milieu in which a soft tissue infection occurs in diabetes. AGE, Advanced glycation endproduct; MP, mononuclear phagocyte; PMN, polymorphonuclear leukocyte; EC, endothelial cell; VCAM-1, vascular cell adherence molecule-1; TF, tissue factor procoagulant activity.

cally relevant ligand; this is especially true of immunoglobulin superfamily molecules, such as intercellular adhesion molecule-1 (ICAM-1).

When RAGE was first identified as a receptor for AGEs, it was found that lactoferrin also bound to AGEs, as well as to RAGE (refs 12, 17). In fact, on certain cell types, cell surface RAGE appears to be complexed with lactoferrin and/or a lactoferrin-like polypeptide. Studies showing that AGEs bind to macrophage scavenger receptors[13] and other polypeptides[11] present on the cell surface lend support to the hypothesis that a common structure present in AGEs might interact with a motif shared by these 'acceptor' molecules. The work by Li *et al.*[1] has provided a concrete step in this direction by defining a cysteine-bounded, hydrophilic motif in lactoferrin and lysozyme that appears to mediate the binding of AGEs. Binding of AGEs to either of the latter polypeptides antagonized their antibacterial properties, leading the authors to propose that this could contribute to the association of diabetes with increased susceptibility to infection, especially in view of the presence of a similar hydrophilic motif in other antimicrobial proteins.

Taken together, these data suggest the following milieu in which a soft tissue infection occurs in diabetes (Fig. 2). Microbial invasion is superimposed on an interstitium and microvasculature rich in AGEs, both those immobilized in extracellular matrix and soluble AGEs. The presence of AGEs renders vascular endothelium hyper-responsive to inflammatory cytokines[12], diminishes the activity of antibacterial hydrophilic polypeptides released from neutrophils[1] and serves to attract, retain and chronically activate mononuclear phagocytes[10-12]. Each of these factors favours extensive tissue destruction rather than containment and resolution of the infection. This resembles a two-hit model in which the sustained presence of AGEs places the host at a disadvantage in dealing with future environmental challenges, in this case, infection. The situation may be particularly devastating when viewed in the context of other AGE-independent mechanisms which have also been implicated in the enhanced susceptibility of patients with diabetes to infection (see table).

What approaches are there for dealing with AGEs in pathologic settings? Based on the considerations described above, one could conclude that considerable clinical benefit would accrue by either blocking preformed AGEs and/or preventing further non-enzymatic glycation. In this context, we have found that the extracellular domain of RAGE (soluble RAGE) binds avidly to AGEs and seems to render them biologically inert with respect to interaction with target cells[12]. It is likely that peptides or other structures modelled on the motif identified by Li *et al.*[1] might also bind AGEs, preventing their interactions with certain polypeptides, such as the hydrophilic antimicrobial proteins. Alternatively, aminoguanidine, an admittedly c mplex agent with multiple activities, has the capacity to limit AGE formation and is in clinical trials for possible beneficial effects on diabetic complications[11].

From this discussion of AGEs, one is likely to draw the conclusion that their mischief takes many forms, resulting in disturbances to a range of critical cellular targets. With the availability of reagents to block AGE-protein/cellular interactions, and to prevent formation or induce degradation of AGEs, it can be anticipated that cause–effect relationships will now be more readily dissected and defined. On the one hand, it is tempting to speculate, as we and others have, that AGEs reflect cumulative changes in macromolecules that occur during ageing, thereby modulating properties of extracellular matrix and perturbing cellular functions and consequently placing the host in a disadvantageous position with respect to combating stressful situations. Alternatively, highly crosslinked and insoluble AGEs might pr vide a relatively inert way to store over the years

- Alteration in [illegible] Gram-negative [illegible]
- Diminished PMN [illegible]
- Changes in bacterial [illegible]
- AGE-attenuation of the activity of [illegible] neutrophils
- AGE-monocyte RAGE interactions promoting [illegible] accumulation and sustained activation
- Hyper-responsiveness of endothelium in an AGE-rich [illegible] mediators

*Literature citations for the first three entries are summarized in ref. 11 [illegible]

molecules sensitive to wear and tear, and their target cellular receptors may represent a way to further detoxify them.

1. Li, Y-M., Tan, A.X. & Vlassara, H. Antibacterial activity of lysozyme and lactoferrin is inhibited by binding of AGE-modified proteins to a conserved motif. *Nature Med.* 1, 1057–1061 (1995).
2. Ruderman, N., Wiliamson, J. & Brownlee M. Glucose and diabetic vascular disease. *FASEB J.* 6, 2905–2914 (1992).
3. Harrington, C. & Colaco, C.A.L.S. Alzheimer's disease: A glycation connection. *Nature* 370, 247–248 (1994).
4. Miyata, T. *et al.* β_2-Microglobulin modified with AGEs is a major component of hemodialysis-associated amyloidosis. *J. clin. Invest.* 92, 1243–1252 (1993).
5. Yan, S-D. *et al.* Non-enzymatically glycated tau in Alzheimer's disease induces neuronal oxidant stress resulting in cytokine gene expression and release of amyloid β-peptide. *Nature Med.* 1, 693–699 (1995).
6. Vitek, M. *et al.* AGEs contribute to amyloidosis in Alzheimer's disease. *Proc. natn. Acad. Sci. U.S.A.* 91, 4766–4770 (1994).
7. Smith, M. *et al.* Advanced maillard reaction end-products are associated with Alzheimer disease pathology. *Proc. natn. Acad. Sci. U.S.A.* 91, 5710–5714 (1994).
8. Palinski, W. *et al.* Immunological presence of AGEs in atherosclerotic lesions of euglycemic rabbits. *Arteriosci. Thromb. Vasc. Biol.* 15, 571–582 (1995).
9. Kirstein, M. *et al.* AGE induces transendothelial human monocyte chemotaxis and secretion of platelet-derived growth factor: Role in vascular disease of diabetes and aging. *Proc. natn. Acad. Sci. U.S.A.* 87, 9010–9014 (1990).
10. Schmidt, A-M. *et al.* Regulation of h mononuclear phagocyte migration by ce face binding proteins for AGEs. *J. clin. Inve* 2155–2168 (1993).
11. Vlassara H., Bucala, R. & Striker L. Pathoger fects of AGEs: Biochemical, biologic, and cl implications for diabetes and aging. *Lab. I* 70, 138–151 (1994).
12. Schmidt, A-M. *et al.* Cellular receptors for A Implications for induction of oxidant stres cellular dysfunction in the pathogenesis of cular lesions. *Arterioscler. Thromb.* 14, 1521-. (1994)
13. Khoury, J. *et al.* Macrophages adhere to gluc modified basement membrane via their s enger receptors. *J. biol. Chem.* 269, 10197–1C (1994).
14. Schmidt, A-M., Hori, O., Chen, J., Brett, J Stern, D. AGE interaction with their endoth receptor induces expression of VCAM-1: A tential mechanism for the accelerated vascul thy of diabetes. *J. clin Invest.* 96, 1375–1 (1995).
15. Li, H., Cybulsky, M., Gimbrone, M. & Lib P. An atherogenic diet rapidly induces VCA 1, a cytokine regulatable mononuclear leu cyte adhesion molecule, in rabbit aor endothelium. *Arterioscler. Thromb.* 13, 197–2 (1993).
16. Hori, O. *et al.* RAGE is a cellular binding site amphoterin: Mediation of neurite outgrow and co-expression of RAGE and amphoterin the developing nervous system. *J. biol. Chem.* (the press).
17. Schmidt, A-M. *et al.* The endothelial binding s for AGEs consists of a complex: An integr membrane protein and a lactoferrin-li polypeptide. *J. biol. Chem.* 269, 9882–988 (1994).
18. Marhoffer, W., Stein, M., Maeser, E. & Federli K. Impairment of polymorphonuclear leukocy function and metabolic control for diabete *Diabetes Care* 15, 256–260 (1992).

Departments of Surgery, Pathology
Physiology and Medicine
Columbia University College of
Physicians and Surgeons
New York, New York 10032, USA

Constraining the cell cycle: Regulating cell division and differentiation by gene therapy

Control of cancer by gene therapy will require choosing the right genes (1052–1056).

DAN T. STINCHCOMB

In the past few years, our knowledge of the regulation of the mammalian cell cycle has burgeoned. Numerous cyclins and their cohorts, the cyclin-dependent kinases, have been identified whose ebb and flow control the progression past various checkpoints in the cell cycle. A growing number of cyclin-dependent kinase inhibitors (hereafter referred to as CDKIs) in turn control the activity of the cyclin-dependent kinases[1]. But how do the CDKIs relate to the cell-cycle dysregulation apparent in actively proliferating tumours? Yang *et al.*, in this issue of *Nature Medicine*[2], demonstrate that a viral vector containing a gene encoding one CDKI, p21, can inhibit tumorigenesis *in vivo*. Expression of p21 in the tumour cells not only inhibits cell proliferation, but simultaneously induces cell differentiation. This paper, in concert with the detailed studies of p21 expression and function already in the literature, demonstrates that p21 is a classic master-switch gene, regulating the decision to divide or differentiate (see figure). Thus, p21 is one of the constraints the human organism places on cell proliferation; manipulating its activity may provide a logical means of treating neoplasia.

p21 is the nexus between the regulatory circuits of the notorious tumour suppressors, *p53* and *Rb*. p53, whose activity can be induced by DNA damage, in turn induces expression of p21 (ref. 3). The CDKI activity of p21 then inhibits the activities of several cyclin-dependent kinases, preventing the phosphorylation of the Rb protein, pRb. When phosphorylated, pRb is inactivated and can no longer sequester the transcription factors of the E2F family. E2F transcription